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Immunoglobulin Attenuates Streptokinase-Mediated Virulence in *Streptococcus dysgalactiae* Subspecies *equisimilis* Necrotizing Fasciitis

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Background. Necrotizing fasciitis (NF) retains a very high mortality rate despite prompt and adequate antibiotic treatment and surgical debridement. Necrotizing fasciitis has recently been associated with *Streptococcus dysgalactiae* subspecies *equisimilis* (SDSE).

Methods. We investigated the causes of a very severe clinical manifestation of SDSE-NF by assessing both host and pathogen factors.

Results. We found a lack of streptokinase-function blocking antibodies in the patient resulting in increased streptokinase-mediated fibrinolysis and bacterial spread. At the same time, the clinical SDSE isolate produced very high levels of streptokinase. Exogenous immunoglobulin Gs (ex-IgGs) efficiently blocked streptokinase-mediated fibrinolysis in vitro, indicating a protective role against the action of streptokinase. In vivo, SDSE infection severity was also attenuated by ex-IgGs in a NF mouse model.

Conclusions. These findings illustrate for the first time that the lack of specific antibodies against streptococcal virulence factors, such as streptokinase, may contribute to NF disease severity. This can be counteracted by ex-IgGs.

Keywords. bacterial spreading; fibrinolysis; necrotizing fasciitis; pooled human immunoglobulin; *Streptococcus dysgalactiae* subspecies *equisimilis*; streptokinase.

Streptococcus dysgalactiae subspecies *equisimilis* (SDSE or group C/G *Streptococcus*) causes a broad spectrum of human diseases ranging from superficial to invasive infections such as necrotizing fasciitis (NF) and streptococcal toxic shock-like syndrome (STSS) [1], commonly associated with *Streptococcus pyogenes* (aka group A *Streptococcus* or GAS). However, recent studies indicate that SDSE has emerged among the bacterial strains isolated from severe infections [2, 3], such that a better understanding of the interplay between molecular pathogenesis of SDSE virulence and host defence strategies is essential.

Necrotizing fasciitis is the most feared severe invasive infection caused by SDSE. The lethality can be as high as 33% [4]. State-of-the-art NF therapy currently consists of surgical debridement, administration of cell-wall-active [5], and protein-synthesis-inhibiting antibiotics [6]. Exogenous commercially available pooled human intravenous immunoglobulin (ex-IgGs or IVIG) may be administered in addition, albeit evidence from sufficiently powered clinical trials is lacking [7]. A comparative observational study recently showed that ex-IgGs infusion is beneficial in patients with GAS STSS [8], suggesting that antibody-mediated blocking of STSS-associated virulence factors (VFs) could prove to be beneficial.

Streptococcus dysgalactiae subspecies *equisimilis*-associated NF is an emerging problem with clinical features reminiscent of GAS-associated NF [1]. In view of these similarities, the repertoire of contributing VFs expressed by SDSE might overlap with those of GAS. In GAS, VFs contributing to invasive infection include the hyaluronic acid (HA) capsule, the pore-forming toxin streptolysin O (SLO), the fibrin-degrading enzyme streptokinase, the cysteine protease SpeB, extracellular deoxyribonucleases (DNases), and the interleukin (IL)-8 cleaving *S pyogenes* cell envelope proteinase (SpyCEP). Many of these VFs have been directly linked to disease severity [9–11], whereas VFs in SDSE have not been evaluated in depth so far.

In this study, we investigated the interplay between the host and the SDSE strain causing a fulminant and lethal NF, aiming to elucidate the pathophysiology of the disease and further optimize treatment. Examination of host factors revealed an absence of streptokinase-blocking antibodies in the patient's plasma, potentially resulting in facilitated bacterial escape due to the lack of containment within the host's fibrin barriers. At

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the same time, the SDSE NF-isolate was characterized by a highly elevated streptokinase activity causing blood clot lysis and consequent bacterial spreading.

METHODS

Case

A 38-year-old morbidly obese woman with a history of drug and alcohol abuse was brought to the emergency room of the University Hospital of Zurich, Switzerland, because of confusion. On arrival, she rapidly developed septic shock, with the Glasgow coma scale falling from 14 to 8, and progressive cardiopulmonary failure requiring mechanical ventilation, volume, and vasopressor therapy. Her legs were covered with psoriatic lesions, bullae, and necrotic areas. Suspected NF was confirmed by magnetic resonance tomography and supported by massively increased leucocyte and neutrophil counts (41.57 grams/L and 39.84 grams/L, respectively) and elevated C-reactive protein (225 mg/L). Initial empiric antibiotic therapy included piperacillin/tazobactam and clindamycin and was complemented by ex-IgGs and extensive surgical debridement of the necrotic subcutaneous tissue of pelvis, thighs, lower legs, and feet. Necrosis progressed requiring subsequent debridement of the abdominal wall. Despite all efforts, sepsis advanced and she died on day 12 in multiorgan failure. In the necrotic tissue, masses of Gram-positive cocci in chains were observed and identified as SDSE. This SDSE NF-isolate was used for subsequent in vitro and in vivo analyses.

Strains and Growth Conditions

The SDSE NF-isolate was isolated from the NF patient described above and compared with the insertional mutant SDSE:*skg* obtained using the insertion plasmid pVE6007-ska-int [12], as previously described [13]. The invasive GAS serotype M1T1 strain 5448, isolated from a NF patient (GAS wild-type [WT] or GAS NF-isolate) [14], was used together with its derivative animal-passaged strain (GAS AP), overexpressing VFs [12, 15], and the deletion mutants GAS Δ*speB* [16] and GAS Δ*cepA* [10]. All strains were grown in Todd Hewitt Broth (BD) supplemented with 2% yeast extract (THY) at 37°C under static conditions. The SDSE:*skg* was grown with 5 µg/mL chloramphenicol. Overnight culture supernatants (16–20 hours) were harvested by centrifugation and filter-sterilized through a 0.22-µm filter (Merck Millipore Ltd.).

Histology and Staining

Tissues were fixed with 10% buffered formalin. Fixed tissue slides (2 µm) were stained with hematoxylin and eosin, Brown Brenn, or acid Fuchsine-Orange G. Whole slide scanning and photomicrography were performed using a NanoZoomer 2.0-HT digital slide Scanner (Hamamatsu).

Virulence Factors Activity Assessment

Activity of the VFs SLO [17], SpeB [18, 19], Sda1 [11], SpyCEP [10], and hyaluronic acid content [20] were assessed as previously described. Streptokinase activity was quantified by assessing the fibrinolytic capacity of the strains' supernatants. Twenty microliters of bacterial supernatant were added to 100 µL plasminogen (20 µg/mL; Haematologic Technlogies) in Tris-buffered saline, pH 7.5. After a 15-minute incubation at 37°C, the generation of plasmin was quantified in a kinetic colorimetric assay (30 minutes, 5-minute intervals, absorbance at 405 nm) with a spectrophotometer (Molecular Devices) using 2.5 mM Spectrozyme (American Diagnostica Gmbh) as substrate.

Strepokinase Sequence Analysis

The streptokinase gene of various clinical SDSE isolates as well as of the GAS serotype M1T1 strain 5448 amplified by polymerase chain reaction was sequenced (Microsynth), and sequence alignment and phylogenetic analysis was performed using MegAlign Pro version 14.1.0 (DNASTAR). A panel of streptokinase GAS sequences was obtained from the National Center for Biotechnology Information for comparison.

emm-Typing

emm-typing of the SDSE NF-isolate was carried out according to the guidelines of the Centers for Disease Control and Prevention (https://www2a.cdc.gov/ncidod/biotech/strepblast.asp). The SDSE NF-isolate was identified as stG6.0 type.

Clotting Assay

Freshly drawn blood was collected in Vacutainer citrate tubes (BD Diagnostics). Platelet-poor-plasma (PPP) was obtained by centrifuging whole blood at 1500 g for 15 minutes, followed by 10 000 g for 10 minutes to remove platelets. When needed, PPP was stripped of the donor's endogenous immunoglobulins (en-IgGs) using Protein-G Gravitrap columns (GE Healthcare). Plasma from 5 healthy human volunteers was screened, and one found displaying susceptibility towards fibrinolysis by SDSE NF-isolate supernatants. To obtain a larger stock of susceptible plasma, consecutive samples of fresh frozen PPP were screened, and the 7th sample was susceptible towards fibrinolysis and used as standard. Clotting assays where performed on a RoTeg analyzer (Axonlab) according to the manufacturer's instructions. Seventy microliters of bacterial supernatant were incubated with either 270 µL blood or 200 µL PPP supplemented with 70 µL fibrinogen (4 mg/mL Haemocomplettan). When indicated, ex-IgGs (11.5 mg/mL Privigen) were preincubated for 20 minutes with bacterial supernatants. To assess clot lysis caused by recombinant streptokinase (rSkg) and to assess the blocking effect of ex-IgGs, PPP was mixed with fibrinogen and purified streptokinase (3 U/mL; Sigma) in the presence or absence of 10 mg/mL ex-IgGs. To assess the specificity of streptokinase activity blockage by ex-IgGs, PPP was mixed with fibrinogen. Recombinant streptokinase (1 U/mL) or SDSE NF-isolate's supernatant were added to the mixture in the presence or absence of 10 mg/mL of a commercial streptokinase-blocking antibody (Abcam ab35168).

Murine Infection Model

The SDSE NF-isolate and SDSE:*skg* were injected subcutaneously (1.5×10^6 colony-forming units) into the shaved flanks of 8- to 10-week-old female C57BL/6 WT mice (Janvier). Bacteria were prepared for injection as previously described [21]. The ex-IgG mice group was treated with intraperitoneal injections of 10 mg of ex-IgGs on day 1 and 5 mg of ex-IgGs on day 2 and 3 postinfection. Mice were sacrificed on day 3 postinfection, and skin samples were collected for enumeration of bacteria [10].

Statistics

Data analysis and presentation were performed using GraphPad software. A 2-sample, 2-tailed, homoscedastic *t* test was used to calculate the indicated *P* values.

Study Approval

Collections of patient and healthy volunteers' samples complied with the current version of the Declaration of Helsinki. The national legal and regulatory requirements and sample collection were approved by the Canton Ethics Committee (Kantonale Ethikkommission Zurich, Switzerland, KEK-ZH-Nr. BASEC 2016-00145 and 2010-0126). For animal studies, the protocol ZH251/14 was approved by the Institutional Animal Care and Use Committee of the University of Zurich.

RESULTS

Histopathological Findings in the Patient

Histopathologic assessment of the debrided necrotic tissue of the NF patient revealed tissue necrosis and fascial acute neutrophilic necrotizing inflammation (Supplementary Figure S1A) as well as the presence of many chain-forming, Gram-positive, partially intracellular cocci (Supplementary Figure S1B) and a striking paucity of extravascular fibrin barriers usually surrounding acute inflammation areas (Supplementary Figure S1C). Furthermore, numerous intravascular fibrin thrombi were present outside of the main lesion (Supplementary Figure S1C, top left panel). The SDSE was isolated from the patient.

Individual Susceptibility to *Streptococcus dysgalactiae* Subspecies *equisimilis* Necrotizing Fasciitis-Isolate-Mediated Fibrinolysis

Given the paucity of extravascular fibrin barriers containing inflamed areas detected in the patient, we examined clotting parameters of the patient and healthy volunteers. Blood from most human donors clotted normally even when spiked with supernatants produced by the SDSE NF-isolate (Figure 1A and C). However, bacterial supernatants induced very efficient clot lysis in a specific subset of donors (Figure 1B and D), similarly to the susceptibility to clot lysis displayed by a sample initially collected from the patient. In addition to promoting clot lysis, SDSE NF-isolate supernatants delayed clot formation and affected maximal clot firmness in susceptible blood phenotypes (Figure 1D), consistent with extravascular fibrin barrier removal at sites of infection.

Elevated Streptokinase Activity in the *Streptococcus dysgalactiae* Subspecies *equisimilis* Necrotizing Fasciitis-Isolate

In a second step, we examined the SDSE NF-isolate for the activity of VFs known to contribute to the pathogenesis of NF caused by the related pathogen GAS, to elucidate their role in the pathogenesis of this particular NF case. The activity of SLO displayed by the SDSE NF-isolate was comparable to that displayed by the GAS NF-isolate, but it was lower than that expressed by its more virulent version GAS AP (Supplementary Figure S2A). No SpeB, DNase, or SpyCEP activities was detectable in the SDSE NF-isolate (Supplementary Figure S2B, C, and D). Hyaluronic acid capsule content was also much lower compared with the GAS NF-isolate (Supplementary Figure S2E). However, the SDSE NF-isolate displayed a higher streptokinase activity compared with the GAS NF-isolate and its AP-derivative (Supplementary Figure S2F). We performed phylogenetic analysis of the streptokinase gene from this SDSE NF-isolate and other SDSE isolates, as well as a panel of GAS streptokinase genes. All SDSE streptokinase genes clustered together, suggesting that there was not a unique phylogenetic origin for the streptokinase gene in this SDSE NF-isolate (Supplementary Figure S3). This SDSE streptokinase cluster was more closely related to streptokinase from M49 GAS NZ131 (Cluster 1 streptokinase) than streptokinase from other GAS isolates.

Endogenous and Exogenous Immunoglobulin Block Streptokinase-Mediated Fibrinolysis

We hypothesized that streptokinase activity inhibition in the resistant blood phenotype samples tested could be attributable to the presence of streptokinase-blocking antibodies. To confirm this hypothesis, en-IgGs were depleted from PPP of individuals resistant to streptokinase-mediated clot lysis. After removal of en-IgGs, addition of SDSE NF-isolate supernatants lead to clot lysis (Figure 2A and C), suggesting that endogenous streptokinase-blocking antibodies were removed. Likewise, addition of pooled human immunoglobulin (ex-IgGs) to PPP derived from streptokinase-susceptible blood resulted in inhibition of clot lysis (Figure 2B and D), indicating that streptokinase-blocking antibodies had been lacking in these susceptible individuals.

Streptokinase Is Necessary and Sufficient to Cause Fibrinolysis in Susceptible Individuals

To test whether SDSE streptokinase activity was necessary to cause fibrinolysis in susceptible blood samples, we generated the SDSE:*skg* strain, lacking streptokinase activity (Supplementary Figure S4A and B). Consistent with streptokinase requirement, fibrin clots were lysed by supernatants of the SDSE NF-isolate but not of SDSE:*skg* (Figure 3A and B). To test whether streptokinase was sufficient to induce clot lysis, exogenous rSkg was added to susceptible and nonsusceptible PPP. Again, rSkg lysed clots in susceptible individuals, and clot lysis was blocked by addition of ex-IgGs (Figure 3C and D) or a commercial streptokinase-blocking antibody

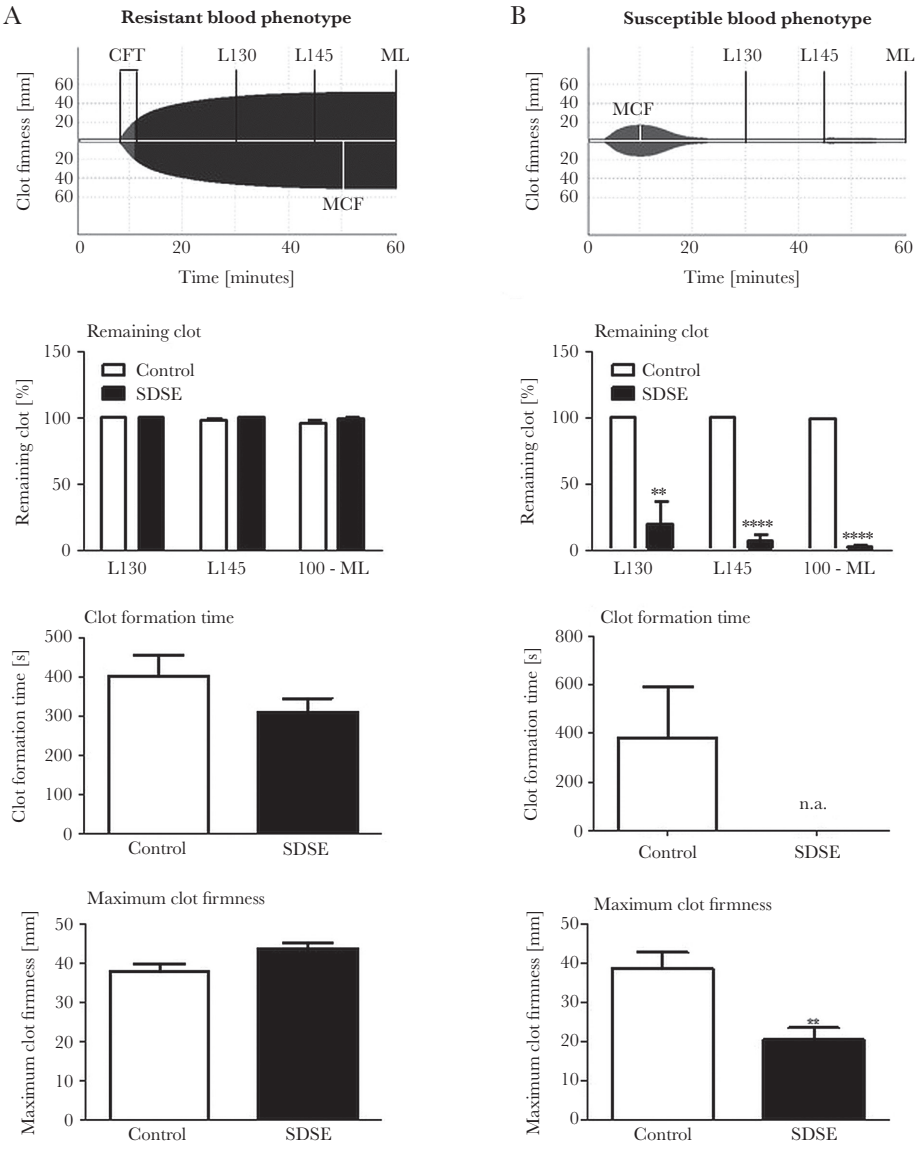

Figure 1. Individual susceptibility to the *Streptococcus dysgalactiae* subspecies *equisimilis* (SDSE) necrotizing fasciitis (NF)-isolate-mediated fibrinolysis. Whole blood from resistant or susceptible donors was mixed with SDSE NF-isolate supernatants, and clot formation and lysis were analyzed using a roTeg analyzer. (*A*) Thromboelastogram of resistant blood phenotype. (*B*) Thromboelastogram of susceptible blood phenotype. (*C*) Clot and lysis parameters of resistant blood phenotype. Values are calculated by pooling the results of experiments from 4 different resistant donors. (*D*) Clot and lysis parameters of susceptible blood phenotype. Values are calculated by pooling the results of 3 independent experiments carried out on a single susceptible donor. Mean values are reported ± standard error of the mean. Significance was calculated using the paired *t* test (**, $P < .01$; ****, $P < .001$). Abbreviations: CFT, clot formation time; LI30, lysis index at 30 minutes; LI45, lysis index at 45 minutes; MCF, monocyte chemoattractant protein; ML, maximum lysis; n.a., infinite clot formation time for weak clots (maximum clot firmness <20 mm).

(Supplementary Figure S5A and B). Blood clot lysis was also blocked by a commercial streptokinase-blocking antibody (Supplementary Figure S5C and D), indicating that streptokinase is necessary and sufficient to mediate clot lysis in susceptible plasma samples.

Blockage of the Virulence Factor Streptokinase by Ex-Immunoglobulin Gs in a Murine Necrotizing Fasciitis Model

To test whether treatment of SDSE NF with ex-IgGs improves clinical outcome by blocking streptokinase activity and containing SDSE infections, mice were infected subcutaneously with the SDSE NF-isolate and treated or not with ex-IgGs. Three days postinfection, mice were euthanized, lesion size at the site of infection was recorded, and bacterial loads in the infected skin were quantified. Although the bacterial count in the skins was identical, a significantly reduced lesion size was observed in the ex-IgGs-treated group (Figure 4A), underlining the importance of antibody-mediated interference with VFs in bacterial infections and consistent with host fibrin deposit containment of bacterial spread.

In a second step, we tested the contribution of streptokinase to SDSE virulence in vivo. Mice were infected subcutaneously

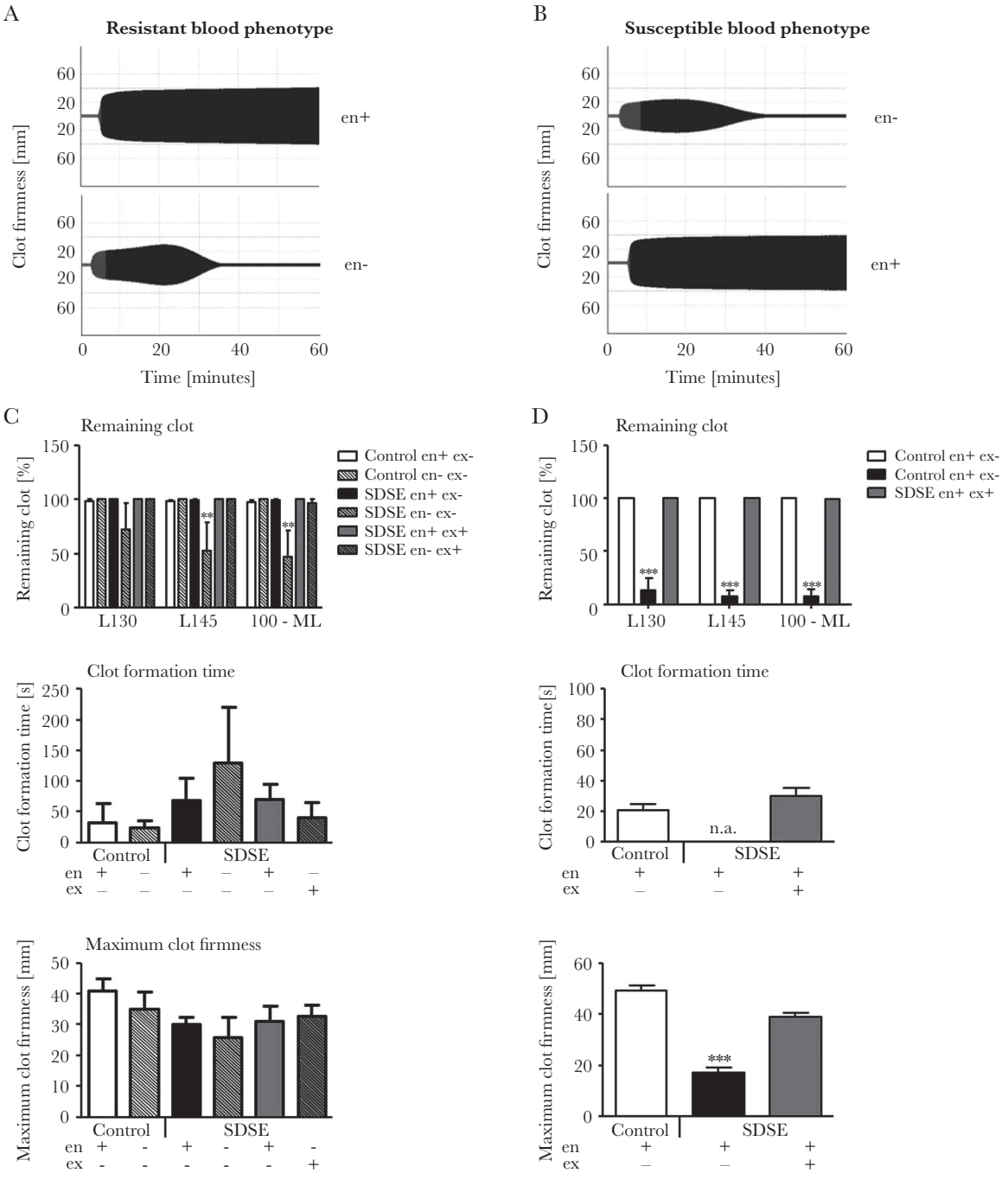

Figure 2. Endogenous and exogenous immunoglobulin (Ig) block fibrinolysis. Platelet-poor plasma from resistant or susceptible donors was mixed with *Streptococcus dysgalactiae* subspecies *equisimilis* (SDSE) necrotizing fasciitis (NF)-isolate supernatants, and clot formation and lysis were analyzed using a roTeg analyzer. (*A*) Thromboelastogram of resistant blood phenotype treated with SDSE supernatant before (top, en+) and after (bottom, en–) depletion of endogenous IgGs (en). (*B*) Thromboelastogram of susceptible blood phenotype treated with SDSE supernatant before (top, ex–) and after (bottom, ex+) addition of exogenous IgGs (ex). (*C*) Clot formation and lysis parameters of resistant blood phenotype before and after depletion of endogenous IgGs (en), with and without exogenous IgGs (ex) addition. The values were calculated by pooling the results of 3 independent experiments on 3 different resistant donors. (*D*) Clot formation and lysis parameters of susceptible blood phenotype before and after addition of exogenous IgGs (ex). Values were calculated by pooling the results of 3 independent experiments on a single susceptible donor. Mean values are reported ± standard error of the mean. Significance was calculated using the paired *t* test (**, $P < .01$; ***, $P < .005$). Abbreviations: CFT, clot formation time; LI30, lysis index at 30 minutes; LI45, lysis index at 45 minutes; ML, maximum lysis; n.a., infinite clot formation time for weak clots (maximum clot firmness <20 mm).

with the SDSE NF-isolate or SDSE:*skg*, devoid of streptokinase activity. Bacterial load was comparable for both strains (Figure 4B). However, in the absence of streptokinase, the lesion size was significantly reduced and consistent with a role of functional streptokinase promoting bacterial spread and causing increased lesion size.

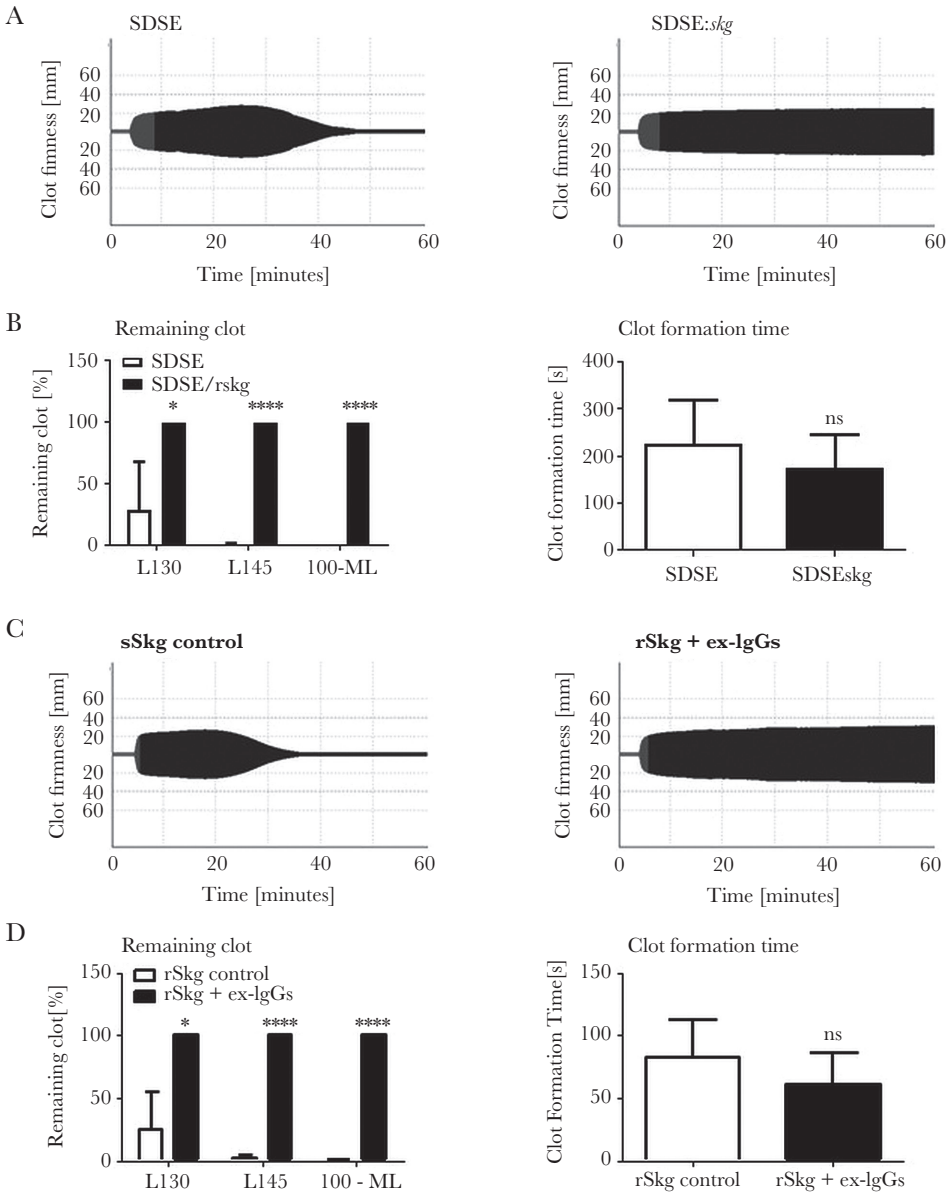

Figure 3. Streptokinase is necessary and sufficient to cause fibrinolysis in susceptible individuals. Platelet-poor-plasma (PPP) from resistant or susceptible donors was mixed with *Streptococcus dysgalactiae* subspecies *equisimilis* (SDSE) necrotizing fasciitis (NF) isolate or SDSE:*skg* supernatants or with recombinant streptokinase (rSkg) in the presence or absence of exogenous immunoglobulin Gs (ex-IgGs). Clot formation and lysis were analyzed using a roTeg analyzer. (*A*) Thromboelastogram of susceptible blood-derived PPP in the presence (SDSE, top left panel) or absence (SDSE:*skg*, top right panel) of streptokinase activity. (*B*) Clot formation and lysis parameters of susceptible blood-derived PPP in the presence or absence of streptokinase activity. (*C*) Thromboelastogram of susceptible blood-derived PPP treated with rSkg in the absence (rSkg control, left panel) or presence (rSkg + ex-IgGs, right panel) of ex-IgGs. (*D*) Clot formation and lysis parameters of susceptible blood-derived PPP in the presence or absence of ex-IgGs. Values were calculated by pooling the results of 3 independent experiments on a single susceptible donor. Mean values are reported ± standard deviation. Significance was calculated using the unpaired *t* test (*, $P < .05$; ****, $P < .001$). Abbreviations: CFT, clot formation time; LI30, lysis index at 30 minutes; LI45, lysis index at 45 minutes; ML, maximum lysis; ns, nonsignificant. rSkg, 3 U/mL final concentration; ex-IgGs, commercial pooled human intravenous Ig (10 g/mL final concentration).

DISCUSSION

Focusing on the interplay between host and pathogen to understand the extensive, rapidly progressive, and lethal case of NF caused by SDSE, we showed that the patient lacked streptokinase-blocking antibodies and that the SDSE strain expressed very high levels of streptokinase. Using genetical and pharmacological loss and gain of function tools, we showed that the presence of streptokinase was necessary and sufficient (1) for causing fibrinolysis in human blood ex vivo in susceptible individuals and (2) to drive virulence in vivo in a murine NF model. The presence of streptokinase was counteracted by antibodies both ex vivo and in vivo. When streptokinase-blocking antibodies were present, fibrinolysis was not observed in human blood ex vivo. Moreover, treatment with pooled human

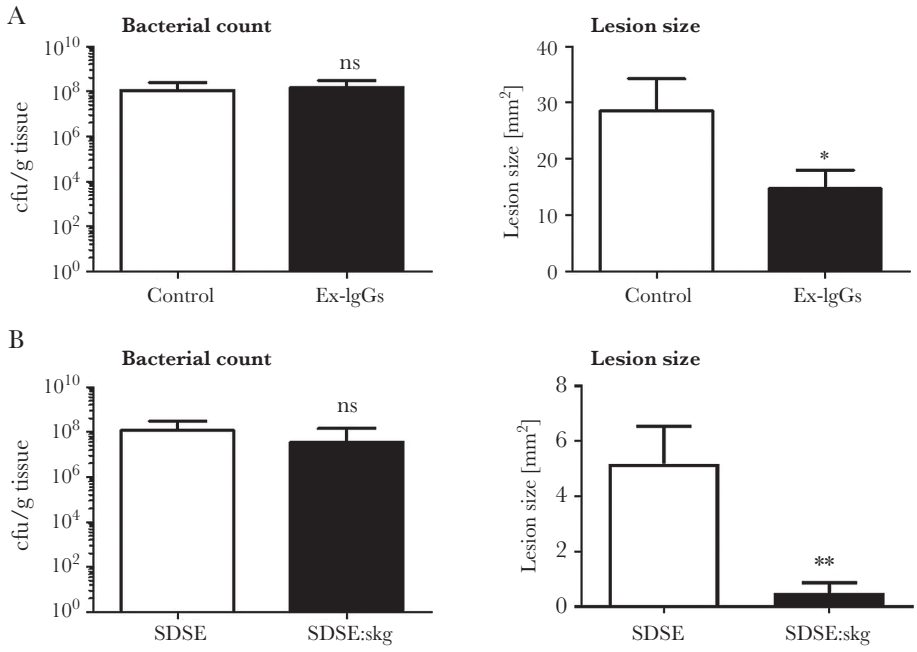

Figure 4. Blockage or absence of streptokinase activity leads to a better clinical outcome. (*A*) C57BL/6 wild-type mice were infected subcutaneously with the *Streptococcus dysgalactiae* subspecies *equisimilis* (SDSE) necrotizing fasciitis (NF) isolate and treated or not treated with exogenous immunoglobulin (ex-lgGs). (*B*) C57BL/6 wild-type mice were infected subcutaneously with the SDSE NF-isolate or the insertional mutant devoid of streptokinase activity (SDSE:*skg*). Three days postinfection, mice were euthanized and bacteria in the skin was enumerated. Lesions size at the site of infection was measured 3 days postinfection. (*A–B*) Data were pooled from 3 and 2 independent experiments, respectively, using 4 and 5 mice/group, respectively, and presented as mean ± standard error of the mean. Significance was calculated using the unpaired *t* test (*, $P < .05$; **, $P < .01$). Abbreviations: cfu, colony-forming units; ns, nonsignificant.

immunoglobulin ameliorated the outcome of the infection in an NF mouse model.

In recent years, the emergence of SDSE-associated invasive infections has been reported [2, 3]. Invasive infections caused by streptococci progress very rapidly, and the lethality associated with such infections, despite state of the art therapy, remains elevated. To dissect the pathophysiology of severe SDSE-associated NF, we started from the clinical observation of lack of fibrin barriers containing acute inflammation areas in the patient's histology. Although some bacteria, such as *Staphyloccoccus aureus*, counteract containment and fibrin wall formation via the virulence factor staphylothrombin, streptococci produce streptokinase, efficiently lysing clots [21]. We speculated that the clinical severity observed in this specific patient was, at least in part, due to the dissolution of fibrin barriers, promoting bacterial spread along the fascia. In a first step, we examined the interplay between the bacterial VF streptokinase and function-blocking host antibodies. We found that the patient's blood and blood from a small subset of healthy volunteers lacked the capacity of blocking clot lysis triggered by SDSE NF-isolate supernatants. On the contrary, blood drawn from most healthy volunteers tested was resistant to the SDSE NF-isolate supernatants-mediated clot lysis. This was a first indication that the patient, and the small subset of healthy donors, might lack a factor able to block an important VF secreted by SDSE and thus be at risk for severe disease.

In a second step, we analyzed the characteristics of the pathogen by assessing the activity of streptococcal VFs, other than streptokinase, reported to contribute to disease severity in streptococcal NF. The pore-forming toxin SLO binds to cholesterol molecules present on the eukaryotic cell membrane to create pores and cause cell lysis [22]. The SLO was expressed at a similar level in the SDSE and GAS NF-isolates. The activity of SpeB [23], which degrades and inhibits many different substrates including human immunoglobulin [24], the complement factor C3 [25–27], and GAS' own VFs [15], was lacking in the SDSE NF-isolate. Despite the importance of DNase in GAS virulence [12, 28], we found the SDSE NF-isolate to be clinically highly virulent despite the absence of DNase activity. Similarly, the IL-8-cleaving enzyme SpyCEP, interfering with host neutrophil recruitment [10], was absent in the SDSE NF-isolate as was the case for hyaluronic acid capsule expression, important in GAS for bacterial adherence [29]. In contrast to GAS, the SDSE NF-isolate expressed high levels of streptokinase. Streptokinase is an enzyme involved in the binding and activation of plasminogen to plasmin, promoting fibrinolysis. During infection, however, streptococci counteract containment of the infection normally mediated by host's formation of fibrin deposits around the site of infection. Enzymatic fibrinolysis is not restricted to *Streptococcus* bacterial species but also present in fungal pathogens such as *Candida* [30]. Because of streptokinase-mediated fibrinolysis, plasmin can also accumulate on

the bacterial surface degrading key host antimicrobial peptides such as LL-37 [21, 31]. The importance of streptokinase activity for virulence was previously shown in a murine GAS model [21, 32] as well as that the variability of streptokinase-mediated plasminogen activation influences virulence and disease outcome [32–34]. We reasoned that, given the absence of fibrin barriers surrounding inflamed areas in the patient's tissue and given the prominent streptokinase production in the SDSE NF-isolate, the lack of streptokinase-blocking antibodies in the host would result in bacterial spreading and ultimately the extreme clinical manifestation observed.

In accordance, depletion of en-IgGs from resistant blood phenotype resulted in blood clot lysis mediated by SDSE supernatants, and addition of ex-IgGs to susceptible blood phenotype resulted in blockage of clot lysis ex vivo. As a proof of principle, ex-IgGs efficiently blocked blood clot lysis mediated by rSkg.

Consistently, in a NF mouse-model, the lesions caused by the SDSE NF-isolate were significantly reduced by treatment with ex-IgGs. Due to the lack of prospective randomized clinical trials, treatment of NF with ex-IgGs is still discussed controversially. However, a recent observational study [8] showed that treatment with ex-IgGs results in clinical benefit in STSS outcome, corroborating previous work showing that streptococcal toxins are blocked by ex-IgGs in cell culture [35–38]. Our case and the associated mechanistic studies provide theoretical support for a therapeutic role of ex-IgGs in NF caused by SDSE. The ex-IgGs efficiently blocked fibrinolysis, as shown ex vivo in PPP, by blocking streptokinase activity. Moreover, lack of streptokinase or blockage by ex-IgGs resulted in in vivo benefits in our murine NF model. Nevertheless, our index patient succumbed, despite receiving ex-IgGs in addition to surgery and all recommended therapeutic options, including cell wall active antibiotics and bacterial protein synthesis inhibitors. However, the patient presented at an extremely advanced stage of disease and in addition had a high number of comorbidities. The potential selection bias in which patients who are the most ill receive therapy with ex-IgGs may underline some of the controversial data found on ex-IgG efficacy in NF patients [7, 39–42]. Consistent with a role of streptokinase activity in bacterial spreading, we observed that in the NF mouse model, wound lesions were significantly larger in animals infected with the streptokinase-expressing SDSE NF-isolate compared with its streptokinase-deficient counterpart SDSE:*skg*.

CONCLUSIONS

We conclude that concomitant expression of high levels of streptokinase by the SDSE NF-isolate and the lack of streptokinase-blocking antibodies in the patient strongly contributed to disease severity, stressing the importance of the interaction between host and bacterial pathogen. Based on our human ex vivo and in vivo murine studies, we conclude that infused ex-IgGs might compensate for the lack of streptokinase-blocking antibodies. Therefore, the use of ex-IgGs as an adjunctive therapy to antibiotics and surgical debridement merits further investigation.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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